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BRIEF REPORT

Plasma Neuropeptide Y (NPY) Increases in Humans in Response to the α_2 Antagonist Yohimbine

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Previous studies have shown that the intravenous administration of yohimbine, an α_2 antagonist, increases norepinephrine turnover and has related anxiogenic effects in humans. We herein report that yohimbine also increases plasma neuropeptide Y (NPY) in healthy human subjects. This finding is consistent with previous reports in animals, but contrasts with a previously reported study in humans. NPY is a 36 amino acid peptide neurotransmitter located in sympathetic and nonsympathetic nerve fibers, as well as in brain structures such as the locus coeruleus, where it is co-localized with norepinephrine. NPY has been shown to inhibit locus coeruleus neuronal firing, decrease norepinephrine release, and increase postsynaptic noradrenergic signal transduction. When administered centrally, NPY also has anxiolytic properties. This study therefore suggests that yohimbine challenge may be useful in assessing NPY and noradrenergic system interactions in neuropsychiatric disorders such as panic disorder or post traumatic stress disorder in which noradrenergic system dysfunction has been observed.
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KEY WORDS: *Yohimbine; Neuropeptide Y (NPY); Human; Noradrenergic*

Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter located in most sympathetic nerve fibers, as well as in nonadrenergic perivascular, enteric, cardiac nonsympathetic, and parasympathetic nerves (Wahlestedt and Reis 1993). NPY is also found in brain structures such as the amygdala, cortex, hippocampus, periaqueductal grey, and locus coeruleus, where it is co-localized with norepinephrine (Heilig and Widerlov 1990), a neurotransmitter thought to be involved in the neurobiology of stress and anxiety disorders. In the locus coeruleus, NPY potentiates α_2-adrenoceptor-mediated inhibition of neuronal firing (Illes and Regenold 1990). NPY has also been shown to decrease the release of norepinephrine and enhance activation of postsynaptic neurons via receptor-mediated increases in Ca^{2+} conductance (Colmers and Bleakman 1994). Therefore, as sympathetically-derived NPY is preferentially released in response to high frequency stimulation (Pernow 1988), it appears that NPY functions to homeostatically regulate norepinephrine release, as well as increase the "synaptic gain" of noradrenergic neurotransmission.

Yohimbine is a noradrenergic α_2 antagonist which induces anxiety while increasing plasma norepinephrine and 3-methyl-4-hydroxyphenylglycol (MHPG) in humans (Holmberg et al. 1962; Charney et al. 1982). Given that norepinephrine and NPY are co-localized and co-released from sympathetic neurons, we hypothesized that NPY levels would also increase in response to yohimbine in humans and that baseline NPY would negatively correlate with the peak MHPG response.

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METHODS

Eight healthy male subjects were included in the study (aged 27.9 ± 9.5 years; range: 21.1–43.7 years). They comprised a subset of control subjects from a previously reported study (Southwick et al. 1993). Subjects were administered normal saline or yohimbine (0.4 mg/kg) in a double-blind, random fashion, at 10:00 A.M. Blood samples were obtained at the following timepoints relative to injection: −30, −15, +40, +60, +120, and +180 minutes. The first blood sample was obtained at least 60 minutes after placement of the intravenous catheter. Plasma was stored at −70°C from the time of initial collection. NPY was measured after plasma extraction using a double antibody RIA using ^{125}I-NPY as the tracer. This RIA possesses an assay sensitivity of 20 pg/ml and intra- and inter-assay coefficients of variation of 8% and 10%, respectively (Allen et al. 1991). MHPG was measured by mass spectrometry as previously described (Southwick et al. 1993).

The average of the −30 and −15 minute samples was used as baseline for each condition. A two-way univariate repeated measures analysis of variance was used to assess the effect of condition (placebo vs. yohimbine) and time, as well as their interaction, on the level of plasma NPY. Planned contrasts were used to determine whether differences between the conditions were present at each time point. The peak change for plasma NPY following yohimbine or placebo was measured by subtracting the baseline level from the peak level. The peak change for yohimbine minus the peak change for placebo gave a net peak effect for yohimbine. Correlations between baseline NPY and the percent change in MHPG (measured between baseline and the peak MHPG level for each subject), and between the percent change in NPY and MHPG were assessed on the yohimbine challenge day only.

RESULTS

Plasma NPY was significantly increased after yohimbine, compared to placebo, at +40, +60, +120, and +180 minutes (Fig. 1). NPY peaked at 120 minutes after yohimbine injection for a net peak increase of 45.6%, compared to placebo. Yohimbine also induced a 21.2% net increase in plasma MHPG. There was a positive correlation between the percent change in plasma NPY and

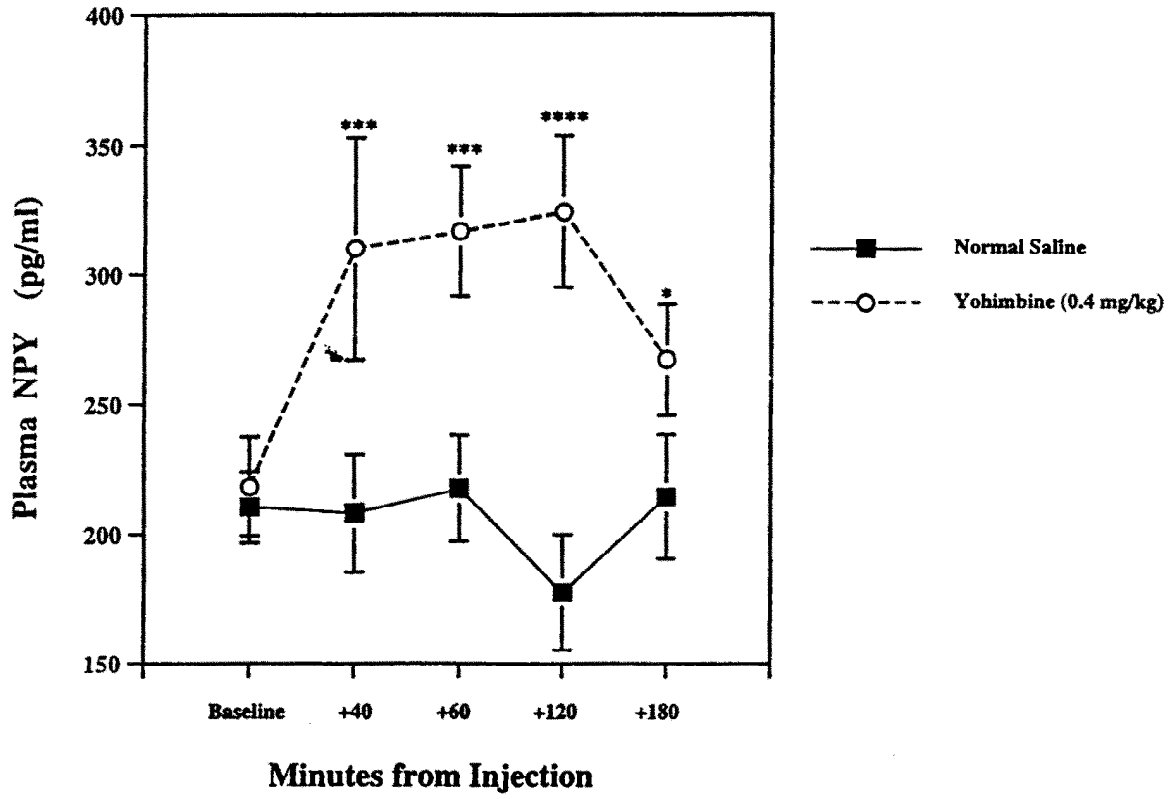

Figure 1. Plasma neuropeptide Y (NPY) in response to the intravenous injection of normal saline or yohimbine (0.4 mg/kg). There was a significant condition effect, $F(1,7) = 13.67$, $p < .008$, and a significant condition by time interaction effect, $F(1,4) = 4.53$, $p < .008$, on plasma NPY levels. Planned contrasts indicated that plasma NPY was significantly increased after yohimbine, compared to the placebo condition, at +40, +60, +120, and +180 minutes after injection: $^{*}p < .05$; $^{***}p < .001$; $^{****}p = .0001$. Each timepoint represents the mean ± the standard error of the mean (n = 6–8).

MHPG ($r = 0.734$, $p < .04$) and a trend toward a negative correlation between baseline NPY and the percent change in MHPG ($r = -0.685$, $p = .061$) on the yohimbine challenge day.

DISCUSSION

Yohimbine previously has been used to elicit a hyperadrenergic anxiety state via antagonism of peripheral α_2 noradrenergic autoreceptors (Holmberg et al. 1962; Charney et al. 1982; Goldberg and Robertson 1983; Henaur et al. 1984). Indeed, in the current study, administration of 0.4 mg/kg yohimbine induced a 21% increase in norepinephrine, as well as a 46% increase in NPY. These findings compare to the results of a previous study in humans by Hedner et al. (1992), in which plasma norepinephrine increased three-fold, while NPY did not significantly change in response to 0.25 mg/kg yohimbine. The discrepancy between studies may be due to differences in the sensitivity and specificity of the NPY radioimmunoassays used (Edvinsson et al. 1990; Allen et al. 1991); also, yohimbine-stimulated NPY release has been shown previously to be dose-dependent (Tavernier et al. 1992). The current findings also support studies demonstrating that plasma NPY is preferentially released in situations of high sympathetic nerve activity (Archeolos et al. 1987; Pernow and Lundberg 1989; Lundberg et al. 1989; Haass et al. 1989; Dahlof et al. 1991; Wahlestedt and Reis 1993), including vigorous exercise (Pernow 1988) and electroconvulsive therapy (Hauger et al. in preparation). The positive correlation between the percent change in NPY and MHPG in response to yohimbine is consistent with the co-release of these co-localized neurotransmitters from peripheral sympathetic neurons. The trend toward a negative correlation between baseline NPY and the percent change in MHPG in response to yohimbine is consistent with previous observations that NPY inhibits the release of MHPG from sympathetic neurons (Colmers and Bleakman 1994).

In a variety of animal models, centrally administered NPY has anxiolytic effects (Heilig et al. 1989, 1993; Wahlestedt et al. 1993). In humans, cerebrospinal fluid NPY levels have been found to correlate negatively with anxiety, but not depressive symptoms (Widerlov et al. 1989). This suggests that NPY may play a role in regulating anxiety, possibly via its effects on the noradrenergic system response to stress.

Previous studies have revealed greater peripheral noradrenergic system responses to yohimbine in patients with panic disorder and posttraumatic stress disorder (PTSD) compared to healthy controls (Charney et al. 1984; Gurguis and Uhde 1990; Charney et al. 1992; Southwick et al. 1993). This raises the possibility that differences in NPY levels or function might mediate or accompany these abnormalities. However, studies of plasma NPY levels in anxiety disorders have thus far yielded discrepant results. In one study, baseline plasma NPY was high in panic disorder patients compared to healthy controls (Boulenger et al. 1996), whereas another study reported baseline plasma NPY to be no different in patients with panic disorder or social phobia compared to healthy controls (Stein et al. 1996). However, given that NPY may play a homeostatic role in regulating the noradrenergic system, further investigation of NPY in the anxiety disorders is warranted and may benefit from a study design in which the noradrenergic system is activated, as for example, by yohimbine challenge or exposure to other defined stressors.

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